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Award Number: DAMD17-02-1-0078

TITLE: Temporal and Spatial Dynamics of DNA Topoisomerase I in Prostate Cancer

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REPORT DATE: January 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
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20050603 056

# REPORT DOCUMENTATION PAGE

*Form Approved  
OMB No. 074-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>			<b>2. REPORT DATE</b> January 2005	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (15 Dec 2003 - 14 Dec 2004)
<b>4. TITLE AND SUBTITLE</b> Temporal and Spatial Dynamics of DNA Topoisomerase I in Prostate Cancer			<b>5. FUNDING NUMBERS</b> DAMD17-02-1-0078	
<b>6. AUTHOR(S)</b> Randy M. Wadkins, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> The University of Mississippi University, Mississippi 38677  <b>E-Mail:</b> rwadkins@olemiss.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b> The purpose of this proposal was to investigate the response of prostate cancer to a series of camptothecin analogs, which are drugs that target DNA topoisomerase I. Toward task 1 of our proposal, we used immunofluorescence staining. However, this did not provide satisfactory results due to non-specificity of the rabbit serum used. We focused predominantly on tasks related to the data obtained from the completed task 3. We developed a very potent camptothecin analog that shows good activity in prostate cancer.				
<b>14. SUBJECT TERMS</b> Topoisomerase I, nucleolus, RNA synthesis, transcription, DNA damage, drug resistance				<b>15. NUMBER OF PAGES</b> 9
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

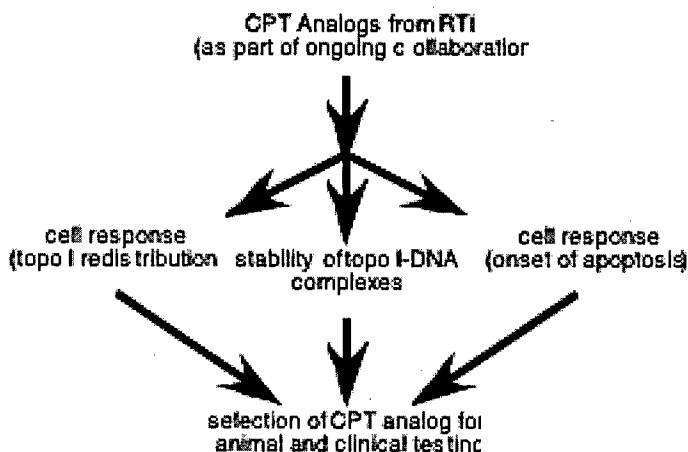
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## INTRODUCTION:

The research proposal is outlined in the flow chart shown in Fig. 1. As part of an ongoing collaboration with Drs. Wani and Manikumar at Research Triangle Institute and Dr. Daniel D. Von Hoff, University of Arizona Cancer Center, we have obtained a number of CPT analogs for analysis against a variety of tumor targets (1). That collaboration was to develop CPT analogs that have increased potency relative to the clinically used topotecan and irinotecan. At present, we have approximately 50 CPT analogs having different topo I inhibition potency and complex stabilization properties. In year 1, we examined this series of compounds in prostate cancer lines to specifically identify compounds likely to be active against prostate cancer. From this work, during year 3, a very potent camptothecin analog (THMAM-MDCPT; see below) was synthesized and tested against PC-3 prostate cancer cells.

Fig. 1. Flow chart for proposed work.



Prostate cancers require special consideration for drugs designed to target them. Consequently, we used three approaches to select analogs that will likely be effective for treatment of prostate cancer. The first assay is that of initial topo I distribution in normal and cancer cells and its response to CPT analog treatment. The second assay is for selection of CPT analogs that form slowly-reversing topo I-DNA complexes. The third is measurement of the onset of apoptosis in prostate cells treated with the different analogs. The outcome of these three assays is then used to select CPT analogs that are most likely to be active in prostate cancer and which should be taken forward to animal studies and potentially clinical trials.

This annual report documents the work performed in the last fully-funded year of the proposal. A no-cost extension for our grant was approved by the DAMD, and the final year of the project will be to use the funds remaining from year 3 to attempt completion of task 1.

## BODY:

### Tasks from Approved Statement of Work

#### Task 1: Measuring Topoisomerase I distribution in prostate cancer and its relationship to response in camptothecins (Months 1-12):

- a. Optimize immunostaining for 6 prostate cancer cell lines being used.(Months 1-4).
- b. Grow normal prostate lines in culture (months 5-8)

- c. Analyze redistribution of topoisomerase I in response to camptothecin analogs (months 8-12).

In year 2, we attempted to develop PC-3 lines that were stably transfected with a fluorescent topoisomerase I (topo I; detailed in last year's annual report), but due to the complex nature of topo I expression, we had to return to immunostaining. This has provided less than satisfactory results for completing task 2. Using rabbit immunoserum means that the reactivity of the serum may vary from lot to lot, and hence we have obtained apparent non-specific immunostaining for topo I. As noted above, we will spend the remaining grant funds to addressing task 1.

**Task 2.** Develop prostate cell lines expressing topo I-EGFP fusion protein (Months 12-18).

- a. Select transfectants of all 6 prostate cancer lines expressing high topo I-EGFP levels (months 12-15).
- b. prepare vectors for knockout/knockin of topo I-EGFP (months 16-18).

Work toward Task 2.

Work toward task 2 has been completed. See last year's annual report for details.

**Task 3.** Measure stability of ternary complexes induced by camptothecin analogs (Months 18-30).

- a. Select analogs with slowest reversal time in purified systems (months 18-24).
- b. Select analogs with slowest reversal time in normal and cancer prostate cells (months 25-30).

Work toward Task 3: The proposed work on Task 3 has been completed (documented in 1st annual report). Camptothecins that form very slowly reversing topo I-DNA complexes have been isolated and directly due to the work from task 3, we have developed 7-trihydroxymethylaminomethyl-10,11-methylenedioxy camptothecin. (THMAM-MDCPT).

A number of new analogs were synthesized bearing increasing hydrogen bond donating groups at the 7-position. These are the mono-, di-, and trihydroxymethylaminomethyl analogs given in Fig. 2, and designated (MHMAM, DHMAM, and THMAM). The corresponding MDCPT analog of each of these was synthesized as well. For comparison, 7-aminomethyl-MDCPT and 7-isopropylaminomethyl-MDCPT were also examined. The results are recorded in Table 1. As shown in Fig. 3, the increasing number of hydroxyl groups on the 7-aminomethyl moiety resulted in progressively more stable cleavable complexes. Whereas our earlier work (2) identified the 10-amino-CPT as the analog that formed the most stable cleavable complex (with a half-life of 17.8 minutes), the 7-THMAM-MDCPT analog showed a remarkably stable complex with a half-life of 116 min.

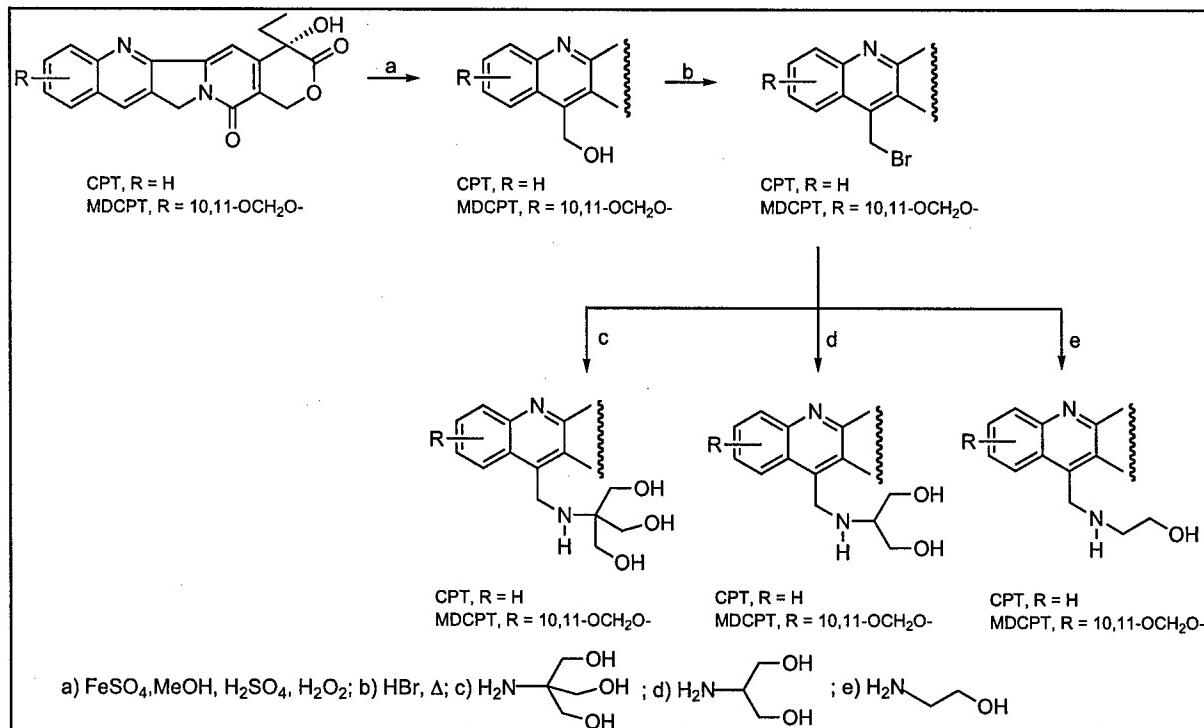


Fig. 2. Synthesis of camptothecin analogs having hydrogen bonding groups at the 7-position.

The addition of the hydrophilic groups at the 7-position did not significantly affect the ability of the CPT analogs to effectively poison topo I in assays with purified enzyme and DNA (compare MDCPT and THMAM-MD in Table 1). Hence the new compounds are very effective topo I poisons. However, one disadvantage of the increased hydrophilicity of the analogs is apparent from their low activity in steady-state culture experiments. The THMAM analog has very low activity in inhibiting cell growth under these conditions. This is presumably due to low cellular permeation by this analog, which is approximately 2 orders of magnitude more water soluble than CPT itself (data not shown). It must be noted, however, that this type of assay is not necessarily predictive of *in vivo* or clinical activity of the compounds (see the data for topotecan in Table 1 for example). These data merely reflect the approximate plasma concentrations that would be necessary to begin to see antitumor activity. Hence, the THMAM-MD and related MD analogs should show activity at concentrations near or lower than those obtained with topotecan or the active metabolite of CPT-11 (SN-38).

**Table 1. Activity of CPT analogs with hydrogen bonding moieties at the 7-position.**

CPT analog	EC <sub>50</sub> for topo I ( $\mu$ M)	k <sub>app</sub> for complex stability (min <sup>-1</sup> )	IC <sub>50</sub> vs. PC-3 (nM)
CPT	0.35 ± 0.01	0.149 ± 0.010	102.8 ± 7.6
SN-38	0.32 ± 0.04	0.061 ± 0.010	25.9 ± 2.1
Topotecan	7.10 ± 4.66	0.169 ± 0.009	281.7 ± 7.1
MDCPT	0.05 ± 0.02	0.058 ± 0.003	12.8 ± 1.0
7-aminomethyl-MD	0.20 ± 0.19	0.051 ± 0.002	19.7 ± 3.0
7-isopropyl- aminomethyl-MD	0.51 ± 0.02	0.047 ± 0.007	19.2 ± 4.0
7-MHMAM-MD	3.00 ± 0.86	0.029 ± 0.007	108.9 ± 40.2
7-DHMAM	4.44 ± 0.49	0.059 ± 0.007	807.3 ± 128.8
7-DHMAM-MD	0.04 ± 0.02	0.023 ± 0.002	206.8 ± 31.5
7-THMAM	2.82 ± 1.86	0.037 ± 0.013	2078.2 ± 405.4
7-THMAM-MD	0.11 ± 0.07	0.006 ± 0.001	90.8 ± 24.5

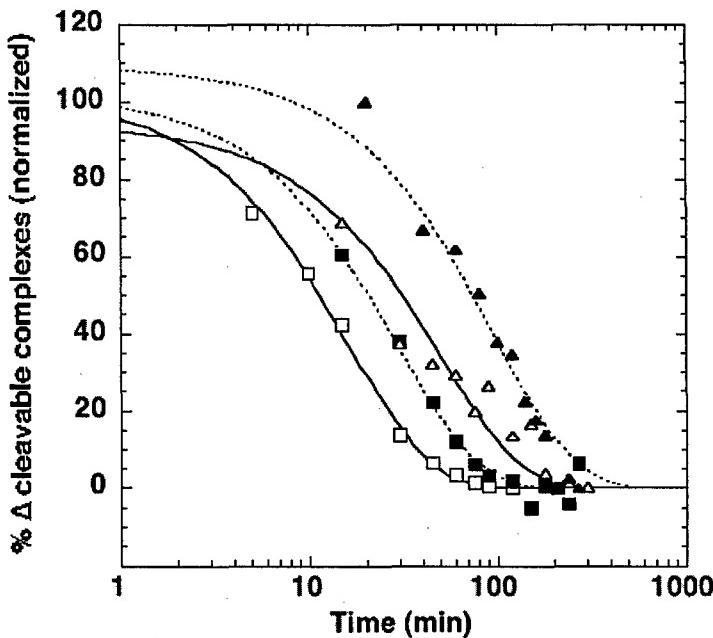


Fig. 3. Stabilization of topo I-DNA complexes by camptothecins. Shown are cleavable complex reversal curves for (□) MDCPT, (■) MHMAM-MDCPT, (△) DHMAM-MDCPT, and (▲) THMAM-MDCPT. As hydroxyl groups are added, considerable slowing of cleavable complex dissociation is observed.

**Task 4.** Measure induction of apoptosis in normal and cancer prostate cells (Months 31-36)

- a. compare induction of apoptosis in rapidly dividing and slow-growing normal and cancer cells (months 31-35)
- b. Correlate apoptosis data with data from tasks 1 and 3 (month 36)

Work toward Task 4.

As Task 4 requires input from Tasks 1-3, we have not begun work on this Task. In the Approved Statement of Work, this Task was to begin during Year 3 of funding. We will begin work on this task during the final year of the project, using the remaining funds from year 3.

**KEY RESEARCH ACCOMPLISHMENTS:**

- Evaluation of camptothecin analogs for activity against prostate cancer cells.
- Development of camptothecin analogs that form stable cleavable complexes.

Wadkins, R. M. "Temporal and Spatial Dynamics of DNA Topoisomerase I in Prostate Cancer

REPORTABLE OUTCOMES:

Wadkins, R. M., Bearss, D., Manikumar, G., Wani, M. C., Wall, M. E., and Von Hoff, D. D. (2004) *Curr. Med. Chem. Anti-cancer Agents* 4, 327-334.

Manikumar, G., Wadkins, R. M., Bearss, D., Von Hoff, D. D., Wani, M. C., and Wall, M. E. (2004) *Bioorg. Med. Chem. Lett.* 14, 5377-5381.

Wadkins, R. M., Bearss, D., Manikumar, G., Wani, M. C., Wall, M. E., and Von Hoff, D. D. (2004) *Cancer Res.* 64, 6679-6683.

CONCLUSIONS:

In this third annual report, the data generated during 2004 is presented, and primarily concerns follow-up data to the completed task 3 of the Approved Statement of Work. We have identified a highly active camptothecin analogs that is active against PC-3 prostate cancer cells. We will use the remaining funds from year 3 during year 4 to accomplish as much of task 1 as possible.

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1. Vladu, B., Woynarowski, J. M., Manikumar, G., Wani, M., Wall, M. E., Von Hoff, D. D., and Wadkins, R. M. (2000) *Mol. Pharmacol.* 57, 243-251.